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SHORT COMMUNICATIONS

Conformational Requirements of *Alpha*$_2$-Adrenergic Receptors

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SUMMARY

The conformationally restrained *trans*-extended and *cis*-folded isomers of 2-(3,4-dihydroxyphenyl)cyclobutylamine were used to establish the conformational requirements of presynaptic *alpha*$_2$-adrenergic receptors in the field-stimulated guinea pig ileum. The *trans*-extended isomer produced a concentration-dependent inhibition of the twitch response with an EC_{50} of 34 μM. The *cis*-folded analogue failed to produce a significant inhibition of the twitch response at concentrations up to 1 mM. These results suggest that the presynaptic *alpha*$_2$-adrenergic receptor prefers phenethylamines in the *trans*-extended conformation over the *cis*-folded conformation. The conformational requirements of *alpha*$_1$- and *alpha*$_2$-adrenergic receptors appear to be similar.

The use of conformationally restrained analogues of norepinephrine has been proposed by Smissman and Gastrock (1) as a possible means of establishing the conformational requirements of adrenergic receptors. These investigators synthesized a series of conformationally restrained norepinephrine analogues that were derivatives of *trans* decalin for this purpose. However, the activity of these rigid norepinephrine analogues has never been evaluated in detail at adrenergic receptors owing to the low agonist activity that accompanied the additional bulk used to restrict conformation.

Erhardt *et al.* (2) evaluated the *alpha*$_1$-adrenergic effects of the *trans*-extended and *cis*-folded isomers of 2-(3,4-dihydroxyphenyl)cyclopropylamine in rabbit aorta. The *trans* form was 5-fold more potent than the *cis* analogue, suggesting that the *trans*-extended conformation, which is the highly preferred conformation in solution and in the solid state (see below), is also the conformation preferred by the *alpha*$_1$-adrenergic receptor.

The study by Erhardt *et al.* (2) represents the most significant advance in understanding the conformational requirements of *alpha*$_1$-adrenergic receptors. However, these investigators did not evaluate the *alpha*$_2$-adrenergic effects of their conformationally restrained phenethylamine analogues. As yet, no data are available concerning the conformational requirements of presynaptic *alpha*$_2$-adrenergic receptors. The present investigation is an attempt to establish the active conformation of β-phenethylamines at the presynaptic *alpha*$_2$-receptor of the guinea pig ileum (3) using the *cis*-folded and *trans*-extended isomers of 2-(3,4-dihydroxyphenyl)cyclobutylamine.

For the evaluation of *alpha*$_2$-adrenergic agonist activity in field-stimulated guinea pig ileum, male albino guinea pigs (640–760 g) were pretreated with reserpine (5 mg/kg, i.p.) 24 hr prior to experimentation, at which time they were killed by a sharp blow to the head. The terminal ileum was removed and cleaned in PSS[3] (pH 7.4) at room temperature and divided longitudinally into segments approximately 30 mm long. All tissues were suspended in 10-ml organ baths containing PSS maintained at 37.5° and aerated with a 5% carbon dioxide-95% oxygen mixture. In all cases, PSS contained cocaine (10 μM), propranolol (1 μM), and sulpiride (1 μM) to block neuronal uptake, *beta*-adrenergic receptors, and dopamine receptors, respectively. This concentration of sulpiride did not significantly alter the EC_{50} of dopamine (*i.e.*, 3.6 ± 0.2 versus 3.9 ± 0.4 μM in control and sulpiride-treated tissues, respectively), indicating that the predominant effect of dopamine in inhibiting the twitch response of this preparation is not at presynaptic dopamine receptors but rather at presynaptic *alpha*$_2$-adrenergic receptors (see below). The tissues were attached to stimulating electrodes for transmural stimulation with the following parameters: 0.8 Hz, 0.01-msec delay, 0.7-msec duration, and 40 V. Under these conditions of field stimulation, neuronally stored acetylcholine is released and activates postjunctional muscarinic receptors to produce a twitch response. Prejunctional *alpha*$_2$-adrenergic receptors, when activated, inhibit stimulation-evoked acetylcholine release and thereby block the twitch response (3).

The results are expressed as means ± standard error of the mean. Statistical differences between two means

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[3] The abbreviation used is: PSS, physiological salt solution (118 mM NaCl, 4.7 mM KCl, 0.54 mM $MgCl_2$, 2.5 mM $CaCl_2$, 1.0 mM NaH_2PO_4, 25 mM $NaHCO_3$, and 11 mM glucose dissolved in demineralized water).

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($p < 0.05$) were determined by Student's t-test for unpaired observations.

All drugs were prepared daily in demineralized water or 0.9% NaCl solution. (−)-Norepinephrine hydrochloride and dopamine hydrochloride were purchased from Sterling-Winthrop (New York, N. Y.) The *cis* and *trans* isomers of 2-(3,4-dihydroxyphenyl)cyclobutylamine were synthesized in the medicinal chemistry laboratories of the Ohio State University and had been used previously to establish the conformational requirements of central dopamine receptors (4).

Dose-response curves for norepinephrine, dopamine, and the *trans*-extended and *cis*-folded isomers of 2-(3,4-dihydroxyphenyl)cyclobutylamine in the field-stimulated guinea pig ileum are presented in Fig. 1. The *trans*-extended isomer of 2-(3,4-dihydroxyphenyl)cyclobutylamine produced a concentration-dependent inhibition of the twitch response with an EC_{50} of 34 μM. The *cis*-folded isomer, on the other hand, was nearly inactive at concentrations up to 1 mM. The EC_{50} values for inhibition of the twitch response in the field-stimulated guinea pig ileum are listed in Table 1.

All studies were performed in the presence of sulpiride (1 μM) to block dopamine receptors. Under these conditions, the effects of norepinephrine, dopamine, and the *trans*-extended analogue were inhibited by yohimbine (1 μM; data not shown), indicating that the effects in Fig. 1 are mediated via presynaptic *alpha*$_2$-adrenergic receptors and not presynaptic dopamine receptors.

Theoretical calculations indicate that the preferred conformation of β-phenethylamines in solution is the extended-*trans* conformation in which the amino and phenyl groups are at a dihedral angle of 180° to each other (5, 6). This conformation also appears to be stabilized by an intramolecular electrostatic or hydrogen-bonding interaction between the amino and β-hydroxyl groups (7). X-ray crystallographic studies of β-phenethylamines in the solid state (8) indicate that, in this state as in solution, the preferred conformation is the extended *trans* form.

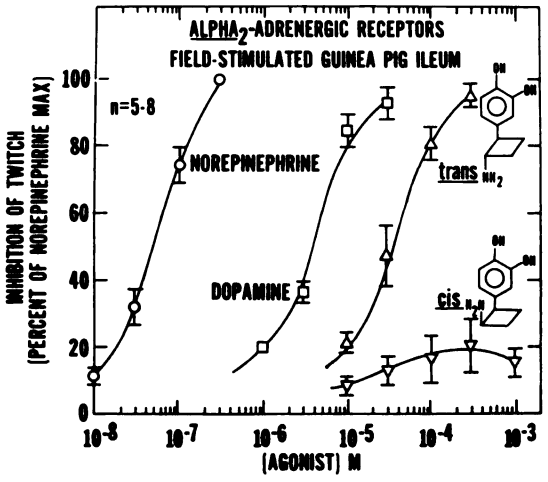

FIG. 1. *Dose-response curves for inhibition of the twitch response in the field-stimulated guinea pig ileum*

This effect is mediated via presynaptic *alpha*$_2$-adrenergic receptors.

TABLE 1

EC_{50} values for inhibition of the twitch response in the field-stimulated guinea pig ileum

Compound	*n*	EC_{50}
		μM
Norepinephrine	8	0.048 ± 0.004
Dopamine	8	3.6 ± 0.17
trans-2-(3,4-Dihydroxyphenyl)cyclobutylamine	5	34 ± 6
cis-2-(3,4-Dihydroxyphenyl)cyclobutylamine	5	>1000

These studies regarding the preferred conformations of β-phenethylamines in solution and in the solid state have led to speculation that the *trans*-extended conformation of β-phenethylamines is required for binding to, and activation of, the *alpha*-adrenergic receptor. Data to support this view are lacking for adrenergic receptors. By using conformationally restrained analogues of dopamine, Erhardt *et al.* (2) provided evidence to suggest that the *trans*-extended conformation is, in fact, that conformation required by the *alpha*$_1$-adrenergic receptor. No results have been published previously in regard to the conformational requirements of *alpha*$_2$-adrenergic receptors.

In the field-stimulated guinea pig ileum, activation of presynaptic *alpha*$_2$-adrenergic receptors inhibits the twitch response. With this system, we have established that the *trans*-extended isomer of 2-(3,4-dihydroxyphenyl)cyclobutylamine is more potent in activating the presynaptic *alpha*$_2$-adrenergic receptor than its *cis*-folded isomer. It is not possible on the basis of these data to establish conclusively whether the greater activity of the *trans*-extended isomer over the *cis*-folded isomer results from a difference in affinity or in intrinsic activity (i.e., efficacy), or both. However, the small response observed with the *cis*-folded isomer in Fig. 1 would tend to suggest that at least the intrinsic activity of the *cis*-isomer is less than that of the *trans*-extended form.

The more active *trans*-extended isomer of 2-(3,4-dihydroxyphenyl)cyclobutylamine is approximately 10-fold less active than the conformationally unrestrained parent compound, dopamine. It is not uncommon for conformationally restrained derivatives to possess lower activity than their nonrestrained counterparts, and this phenomenon has generally been attributed to the additional bulk which is necessarily incorporated into the restrained analogues to restrict their conformation. As such, the more meaningful comparison to be made when conformationally restrained analogues are evaluated is among *stereoisomers* of the rigid analogues. On the basis of such a comparison, the data reported herein using the *trans*-extended and *cis*-folded isomers of 2-(3,4-dihydroxyphenyl)cyclobutylamine strongly suggest that the *trans*-extended conformation of phenethylamines is the conformation preferred by the *alpha*$_2$-adrenergic receptor and this would indicate that the conformational requirements of both *alpha*$_1$- and *alpha*$_2$-adrenergic receptors are similar.

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